ELECTRON MICROSCOPIC COMPARATIVE ANALYSIS OF SMEAR LAYER REMOVAL BY ULTRASONICALLY ACTIVATED AND DIODE LASER ACTIVATED - EDTA AND CHITOSAN - AN INVITRO STUDY

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

In partial fulfilment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH - IV

CONSERVATIVE DENTISTRY AND ENDODONTICS

2016 – 2019

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Date: 21.01.2019

Place: Elayampalayam

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This is to certify that **Dr. VINEETHA.C. S**, Post graduate student (2016-2019) in the **DEPARTMENT OF CONSERVATIVE DENTISTRY AND ENDODONTICS,** Vivekanandha Dental College for Women, has done this dissertation titled **"ELECTRON MICROSCOPIC COMPARATIVE ANALYSIS OF SMEAR LAYER REMOVAL BY ULTRASONICALLY ACTIVATED AND DIODE LASER ACTIVATED - EDTA AND CHITOSAN - AN INVITRO STUDY"** under our guidance and supervision in partial fulfilment of the regulations laid down by the Tamilnadu Dr. M.G.R. Medical University, Chennai-600032 for **M.D.S (Branch-IV) CONSERVATIVE DENTISTRY AND ENDODONTICS.**

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PLACE OF STUDY	**VIVEKANANDHA DENTAL COLLEGE FOR WOMEN**
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CONTENTS

INTRODUCTION

INTRODUCTION

Root canal treatment is an essential procedure carried out in various clinical situations which include teeth with deep caries and irreversible pulpitis, following trauma, attrition, resorption and in certain clinical conditions such as prosthetic rehabilitation of missing teeth, when the tooth/teeth need to be taken as an abutment.

The success of an endodontic procedure majorly lies on 3 important factors that include – Creating a straight line access, proper cleaning and shaping of the canals and producing a 3 Dimensional obturation with a good seal.

Cleaning and Shaping previously described as Biomechanical preparation is one of the most essential step in achieving a successful clinical outcome. It refers to shaping the canal to produce a smooth, continuously tapering canal from the orifice to the apex and disinfecting the canal walls by removal of the dentinal debris, infected pulpal tissue, bacteria and its by-products.

Shaping of the canal wall is carried out using endodontic hand files or rotary instruments by cutting the root dentin along the canal walls. This leads to the production of an irregular amorphous smear layer.

Erick et al identified smear layer using Scanning Electron microscope in 1970s. He stated that “Smear layer contains inorganic debris and organic material. The organic material includes vital and necrotic pulpal tissues, microorganisms and their metabolic products and odontoblastic processes”. The smear layer is divided into two portions –Superficial layer which is present on the surface of the canal wall and a smear plug which is the portion of the smear layer within the dentinal tubules.

This was discussed by Cameron et al[1] and Mader et al[2]. Brannstrom and Johnson estimated the thickness of smear layer to be 2-5 µm in 1974[3].

The controversy regarding retaining or removing smear layer still prevails. Some investigators support the concept of not removing the smear layer because it would seal the tubules thus preventing the entry of bacteria and their by-products, whereas others support the concept of its removal because they believed that the smear layer could act as a scaffold for harbouring bacteria and it would interfere in production of proper seal.

Cleaning the canal refers to the removal of smear layer, which can be done using chemical agents, ultrasonics and/or by the use of laser. Chemical agents such as sodium hypochlorite, EDTA and organic acids in combination with ultrasonics and laser agitation have also been used.

Ethylenediaminetetraacetic acid (EDTA) is the most commonly used irrigant/chelating agent for removal of smear layer[4, 5, 6]. It promotes decalcification by chelating the calcium ions in dentine at approximate depths of 20–30 µm within 5 min[7]. EDTA has harmful effect on periapical tissues and this has led researchers to seek more biocompatible material as an alternative. Weak acids, like apple cider vinegar and citric acid have also been studied [6, 8, 9]. Citric acid reacts rapidly with calcium ions low cytotoxicity[10] and antimicrobial properties[11].

Chitosan has gained attention in dental research because it is biocompatible, biodegradable possesses the property of bio adhesion and lack of toxicity[12, 13]. Chitosan is obtained by the deacetylation of chitin, which is found in crab and shrimp shells[14].

Application of chitosan has been seen in the field of medicine and pharmaceuticals (antibacterial and anti-tumour agent, drug carrier, wound healing accelerator), biotechnology (enzyme and cell carrier, chromatography resin), environment (water treatment), agriculture (seed preparation), cosmetics and food (iron and calcium absorption accelerator, fibre source)[15]. The use of Chitosan as a chelating agent has not been widely discussed. Recently, studies are being carried out to assess the efficacy of Chitosan as an irrigant for its chelating and antibacterial property.

Syringe irrigation is the conventional and still widely used irrigation technique. Various techniques such as moving gutta-percha (GP) cones up and down in the root canal (manual dynamic activation [MDA]) to instruments energized by (ultra)sonic or laser devices have been used for the activation of irrigants. Chemical chelating agents in combination with Ultrasonic agitation and lasers have shown to produce better results than conventional syringe irrigation.

The use of ultrasonics has been proposed as a possible solution to the problem of debriding and disinfecting the root canal system. The use of ultrasound after completion of hand or rotary instrumentation has been shown to reduce the number of bacteria. The cavitational effect produced on the canal walls by Ultrasonic agitation was found to have improved effects in combination with various irrigating solutions.[1]

Recently, the use of laser devices for agitating the irrigating solutions has gained popularity. Several studies have used Nd-YAG lasers for canal disinfection. The advantage of near-infrared diode laser is that the fibre is thin and flexible, which allows access into narrow and curved root canals, and it provides increased

disinfection of the deep radicular dentin. The type of irrigating solution used and the laser wavelength determines the quantity of irrigant absorbed into the canal walls.

One of the effectively used methods to determine the ability of smear layer removal is Scanning electron microscopy. Among the various scoring systems for quantifying the remaining smear layer, Gutmann's scoring criteria was followed in the present study.

In this study, the efficacy of Chitosan and EDTA in combination with Ultrasonic and Diode lasers for agitation was compared and the ability of smear layer removal was evaluated. Hence, the use of novel chelating agents which are biocompatible, with minimal tissue toxicity and better antibacterial efficacy, in combination with newer irrigating systems which would aid in better debridement of the root canal would improve the clinical outcome of root canal treatment.

AIM AND OBJECTIVES

AIM AND OBJECTIVES

AIM OF THE STUDY

The aim of this study is

- To compare the efficacy of Ultrasonically activated and Laser activated EDTA and Chitosan on smear layer removal by using Scanning Electron Microscope

OBJECTIVES OF THE STUDY:

- To evaluate the efficacy of Chitosan on smear layer removal
- To compare the efficacy of Chitosan and EDTA
- To compare the efficacy of Ultrasonic and Diode laser activation of irrigants.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Cameron et al[1] in 1983, studied the smear layer removal efficacy of ultrasonics by scanning electron microscopy.

Yamada et al[16] in 1983, compared EDTA and NaOCl solutions as a final flush with various other solutions, or in combinations, using SEM. He concluded that 10 ml of 17% EDTA buffered to pH 7.7 followed by 10ml 5.25% NaOCI solution had the highest ability of smear layer removal.

Bystrom et al[17] in 1985, compared the efficacy of 0.5% and 5 % NaOCl sodium solutions and concluded that there no significant difference between the antibacterial effects. When combined with EDTA it showed more efficacy.

Baumgartner et al[18] in 1987, evaluated the ability of four irrigation regimens on debridement of root canals. They stated that combination of EDTA and NaOCl removed the pulpal remnants as well as the smear layer.

Ciucchi et al[19] in 1989, compared smear layer removal efficacy by different procedures and concluded that consistently smeared surfaces were produced by NaOCl and the smear layer was moderately removed when NaOCl was stirred using ultrasonics. EDTA almost fully removed the smear layer and the chelating ability was not enhanced when EDTA was combined with ultrasonics.

Aktener et al[20] in 1993, tested the effect of EDTA and ethylenediamine mixtures and concluded that 10 ml of a 4:3 by volume mixture of EDTA and ethylenediamine completely removed the smear layer.

Gutmann et al[21] in 1994, compared the preparations of apical cavities in resected root ends using rotary burs, with and without citric acid rinse, and ultrasonic tips based on the presence or absence of superficial debris and smear layer. In this study root-end preparation with a bur created a heavy smear layer at all levels of the

preparation. This layer was partially removed during ultrasonic preparation in the apical two-thirds. A greater removal of the smear layer was achieved with the citric acid rinse ($P<0.05$). Coronally, root-end preparations were contaminated with moderate to heavy mounts of debris with all techniques.

Prati et al[22] in 1994, manual endodontic instruments - an ultrasonic and an endosonic system were studied with a view to evaluating the morphology of the smear layer and the amount of debris and pulpal residues in the apical third of human extracted straight teeth from 55 to 75 year old patients. The manual instruments were K files, Ergoflex files used with the step-back technique, Canal Master with its own technique, and Flex-R with the Roane technique. The ultrasonic system was Suprasson Piezo and the endosonic was Excalibur. Human extracted teeth with straight canals were used and were examined under a scanning electron microscope. All manual instrumentations showed a homogeneous compact smear layer and no pulp residues. No statistical differences were observed among the four manual techniques. Ultrasonic technique showed the complete removal of the smear layer, leaving small amounts of pulp debris at the apical third, while the Excalibur showed an almost complete elimination of the smear layer, leaving a homogeneous layer of pulpal residues along the canal.

Behrend et al[23] in 1996, determined the effect of smear layer on obturation and stated that the sealing ability and resistance to bacterial penetration was enhanced in the presence of smear layer.

Chailertvanitkul et al[24] in 1996, studied the correlation between the smear layer and canal obturation. He showed that that there was no significant difference in leakage with or without the presence of smear layer as measured by bacterial penetration.

Takeda et al[25] in 1999, compared the ability of three acidic irrigants and two lasers on smear layers formed after hand instrumentation and stated that these irrigating solutions cause demineralisation of inter tubular dentine and Er:YAG laser was the most effective in the removal of the smear layer.

Sabins et al[26] in 2003, compared sonic and passive ultrasonic irrigation and found that ultrasonic passive irrigation produced cleaner canals than hand filing and passive sonic irrigation.

Crumpton et al[27] in 2005, used 17% EDTA with rotary instrumentation to evaluate the effect of additional irrigation on smear layer removal and concluded that a final rinse with 1ml of 17% EDTA for 1 minute produced effective removal of smear layer.

Wang et al[28] in 2005, investigated temperature rise of canal wall during and after irradiation with diode laser and the results showed that diode laser can be effectively used in clinical practice.

Carver et al[29] in 2007, compared the in vivo antibacterial efficacy of a hand/rotary technique versus a hand/rotary/ultrasound technique in mesial root canals of necrotic mandibular molars. The addition of 1 minute of ultrasonic irrigation resulted in significant reduction in CFU count and positive cultures. Logistic regression analysis indicated the addition of ultrasonic irrigation was 7 times more likely to yield a negative culture.

Mozayeni et al[31] in 2009, compared MTAD with 17% EDTA and showed that MTAD as the final rinse was more effective in the apical third, while 17% EDTA was more effective in the middle and coronal third.

Gregorio et al[32] in 2009, evaluated the penetration of 5.25% sodium hypochlorite alone or in combination with 17% EDTA in simulated lateral canals

using sonic and ultrasonic activation. They observed that irrigant penetration into the lateral canals was not enhanced by the addition of EDTA.

Kuah et al[33] in 2009, studied the effect of 17% EDTA with and without ultrasonics. They stated that, a combination of EDTA and ultrasonics effectively removed smear layer from the apical third.

Gu et al[34] in 2009, compared various irrigating solutions after post space preparation to determine the effect of combining ultrasonics and concluded that EDTA was better than NaCl and NaOCl in the removal of smear layer, but addition ultrasonics did not show any significance difference.

Hmud et al[30] in 2009, examined whether near infrared 940 and 980 nm diode lasers (Biolase Ezlase and Sirona Sirolaser, respectively) could induce cavitations in aqueous media. They concluded that both diode laser systems could induce cavitation in water-based media by the formation and implosion of water vapour. Laser power played a more important role than pulse frequency or pulse interval. Optimal laser-initiated cavitation occurred when weak (3%) peroxide solutions were used as the target irrigant, rather than water.

Desai et al[35] in 2009, evaluated the safety of various intracanal irrigation systems by measuring the apical extrusion of irrigant. This study showed that the EndoVac did not extrude irrigant after deep intracanal delivery and suctioning the irrigant from the chamber to full working length. EndoActivator had a minimal, although statistically insignificant, amount of irrigant extruded out of the apex when delivering irrigant into the pulp chamber and placing the tip into the canal and initiating the sonic energy of the EndoActivator. Manual, Ultrasonic, and Rinsendo groups had significantly greater amount of extrusion compared with EndoVac and EndoActivator.

Rodig et al[36] in 2010, compared the efficiency of a sonic device (Vibringe), syringe irrigation, and passive ultrasonic irrigation in the removal of debris from simulated root canal irregularities. They concluded that passive ultrasonic irrigation is more effective than the Vibringe System or syringe irrigation in removing debris. The sonic device demonstrated significantly better results than syringe irrigation in the apical root canal third.

Jiang et al[37] in 2010, evaluated the effect of pulsed ultrasound on passive ultrasonic irrigation (PUI) in its ability to remove artificially placed dentin debris from a simulated apical oval extension within standardized root canals. They concluded that PUI with a pulsation pattern of 400 milliseconds on/400 milliseconds off and a duty cycle of 50% is more effective in removing dentin debris from a simulated apical oval extension in standardized root canals than continuous ultrasonic activation. Duty cycles of 13% and 88% showed no difference compared with continuous oscillation.

Caron et al[38] in 2010, examined the effect of different final irrigation regimens. He concluded that root canal cleanliness benefits from solutions activation (especially sonic activation and manual-dynamic activation) in comparison with no activation during the final irrigation regimen.

Pagonis et al[39] in 2010, studied the in vitro effect of PLGA nanoparticles with photosensitiser against E.fecalis and concluded that these particles encapsulated with photoactive drugs may have antimicrobial properties.

Saber et al[40] in 2011, compared 3 different irrigation activation methods and found that ANP (apical negative pressure) and MDA (manual dynamic agitation), when used for the final irrigant activation produced better results.

Jiang et al[41] in 2011, evaluated the effect of the ultrasonic intensity on PUI to remove dentin debris and whether there was any lateral effect beyond the ultrasonic tip. The results showed that higher ultrasonic intensity resulted in higher amplitude of the oscillating file and, consequently, enhanced the cleaning efficacy of PUI.

Ulusoy et al[42] in 2011, evaluated the root dentine micro hardness smear layer removal efficacy and erosion caused by various irrigants such as maleic acid, EDTA, MTAD and Smear Clear and showed that maleic acid was the most efficacious in the apical third of the canal. It also showed the greatest reduction in microhardness.

Andrabi et al[43] in 2012, compared the smear layer removal efficacies of 3% sodium hypochlorite (NaOCl), 17% Ethylenediaminetetraacetic acid (EDTA), SmearClear and BioPure MTAD using a common irrigation protocol and stated that, in the apical third, MTAD had the highest efficacy.

Pimenta et al[44] in 2012, conducted a study to evaluate the micro harness of root dentine following the use of 0.2% chitosan, 15% EDTA and 10% citric acid and concluded that there were no significant differences between them.

Stojicic et al[45] in 2012, evaluated the ability of QMiX against E.fecalis and showed that a combination of QMiX and NaOCl in killing E.Fecalis was better than combination of chlorhexidine and MTAD. Smear layer removal efficacy of QMiX was similar to that of EDTA.

Castelo-Baz et al[46] in 2012, compared the effects of 2 ultrasonic irrigation techniques on the penetration of sodium hypochlorite into the main canal and simulated lateral canals of extracted teeth. They concluded that Continuous Ultrasonic Irrigation as a final rinse significantly increased the penetration of irrigating solution into simulated lateral canals compared against positive pressure irrigation and passive ultrasonic irrigation techniques.

Jiang et al[47] in 2012, evaluated the removal of dentin debris from artificially made grooves in standardized root canals by 6 different final irrigation techniques i.e. Conventional syringe irrigation, manual dynamic activation (MDA) with tapered or non-tapered gutta-percha (GP) cones, the Safety Irrigator system, continuous ultrasonic irrigation (CUI), and apical negative pressure (ANP).They concluded that CUI was the most effective technique in dentin debris removal from the apical irregularities, and syringe irrigation alone was the least effective. MDA technique was more effective with a tapered GP cone than with a non-tapered one.

Silva et al[48] in 2012, assessed the smear layer removal efficacy of different concentrations of chitosan and studied the dentin structure after application for 3 and 5 min. They concluded that, 3 minute application of 0.2% chitosan produced efficient smear layer removal with little dentine erosion.

Mancini et al[49] in 2013, evaluated the effectiveness of different irrigating methods (EndoActivator, EndoVac, and Passive Ultrasonic Irrigation) in the removal of smear layer at various lengths from the apex of root canals. The results showed that the EndoActivator and EndoVac showed the best results at 3, 5, and 8 mm (EndoActivator) and 1, 3, 5, and 8 mm (EndoVac) from the apex.

Arslan et al[50] in 2013, showed that effective smear layer removal was achieved from the apical third of the root canal, when 15% EDTA was agitated using an 808-nm diode laser for 20seconds.

Silva et al[51] in 2013, evaluated the efficacy of chitosan compared to 17% EDTA, 1% acetic acid and 10% citric acid on smear layer removal using scanning electron microscopy. The results showed that 15% EDTA and 0.2% chitosan had the greatest effect on demineralisation of root dentin.

Kim et al[52] in 2013, investigated the relative efficacies of the flowable gel-type and liquid-type EDTA solutions for removal of the smear layer and inorganic debris. They also evaluated the effects of manual dynamic activation (MDA). The results suggested that gel-type EDTA might be an acceptable irrigant for removing the smear layer and inorganic debris present on the root canal wall.

Gusiyska et al[53] in 2013, demonstrated by SEM analysis, that a 0.6% solution of chitosan in 1% citric acid was very effective at removing the smear layer.

Darrag et al[54] in 2014, compared 4 different irrigating solutions as final rinse on smear layer removal and concluded that 0.2% chitosan solution was more effective than EDTA, citric acid and MTAD.

Persadmehr et al[55] in 2014, evaluated the ability of photodynamic therapy (PDT), chitosan nanoparticles (CSnp), or their combination, to inhibit bacterial collagenase-mediated degradation of collagen. This study showed that collagen treated with CSnp, PDT, or a combination of CSnp and PDT, exhibited less degradation than controls. The abundance of post-treatment residual collagen correlated with the extent of degradation. Fourier transform infrared (FTIR) spectroscopy analysis showed that PDT treatment enhanced collagen cross-linking .Immunoblotting of sedimented CSnp indicated that CSnp and collagenase bound with low affinity. However, CSnp-bound collagenase showed a significant reduction in collagenolytic activity compared with controls.

Neelakantan et al[56] in 2015, investigated the impact of three irrigation protocols, activated by three different methods, on mature biofilms of Enterococcus faecalis in vitro and concluded that the use of NaOCl after or in combination with a chelator caused the greatest reduction of E. faecalis. Diode laser and Er-YAG laser activation were superior to ultrasonics in dentinal tubule disinfection.

Perochena et al[57] in 2015 studied the use of bioactive CNPs on smear layer removal and inhibition of bacterial recolonization. He concluded that CNPs were effective on both inhibiting bacterial recolonization and removal of smear layer.

Amin et al[58] in 2016, evaluated the efficacy of diode laser and ultrasonics with or without EDTA and concluded that Diode laser alone performed significantly better than ultrasonics in the removal of smear layer.

Afkhami et al[59] in 2017, compared the efficacy of silver nanoparticles (AgNPs), an 810-nm diode laser (DL), conventional photodynamic therapy(PDT) with the use of indocyanine green (ICG) photosensitizer, and modified PDT with the use of AgNPs for the disinfection of root canals inoculated with Enterococcus faecalis. The results showed that PDT with ICG, an 810-nm diode laser, and AgNPs have the potential to be used as an adjunct for disinfection of the root canal system.

Machado et al[60] in 2017, compared the efficacy of EDTA and citric acid and concluded that sealer penetration into the dentinal tubules was increased significantly throughout the entire length of the canal whereas the smear layer removal ability of the chelating solution was restricted to middle and coronal third.

Perochena et al[61] in 2017, evaluated the efficacy of chitosan nanoparticles (CNPs) and ethanolic propolis extract (EPE) incorporated into a calcium hydroxide paste ($Ca[OH]^2$) to kill bacterial biofilms. They concluded that incorporating CNPs into pastes of $Ca(OH)^2$ could potentially be beneficial when using inter appointment intracanal medications because of their ability to kill bacteria in short- and long-term exposure.

Simezo et al[62] in 2017, assessed ex vivo the erosive effects of passive ultrasonic irrigation versus irrigation with reciprocating activation on the dentinal surface of the root canal at 3 predetermined levels (3, 6 and 9mm) using

environmental scanning electron microscopy. They concluded that the final irrigation techniques tested were equivalent in relation to the degree of erosion caused to the dentinal surface.

Zhou et al[63] in 2018, compared chitosan and MTAD and concluded that in the apical third, the smear layer removal efficacy of chitosan was better than MTAD.

MATERIALS AND METHODS

MATERIALS AND METHODS

SOURCES OF SAMPLES:

75 mandibular premolars with single canal (Department of Oral and Maxillofacial Surgery, Vivekanandha Dental College for Women).

Materials used:

- Normal saline ***(Claris Otsuka LTD, Ahemedabad, India)***
- 3 % NaOCl irrigating solution ***(VensonsIndia, Bengaluru,India)***
- Low molecular weight Chitosan ***(Sigma Aldrich, Missouri, United States)***
- 17% EDTA ***(Desmear, Anabond Stedman Pharma, Chennai, India)***

Armamentarium:

1. Diamond Disc
2. Straight Handpiece ***(NSK, Japan)***
3. Scale
4. Endoblock
5. Electronic weighing device
6. Hot Plate Magnetic Stirrer
7. Disposable 2ml Syringe ***(Unolok Syringes and Medical Devices LTS, Faridabad, India)***
8. X-Smart Plus Endomotor and Handpiece ***(Dentsply Maillefer, Ballaigues, Switzerland)***
9. K files – ISO size 10, 15 and 20 ***(Mani Inc., Japan)***
10. Protaper files SX, S1,S2, F1, F2, F3 ***(Dentsply Maillefer, Ballaigues, Switzerland)***
11. U File 33mm , 20 ISO (***Mani Inc., Japan)***
12. Ultrasonic unit ***(Woodpecker)***
13. Diode Laser ***(Zolar Photon plus)***
14. Scanning Electron Microscope ***(Carl zeiss)***

METHODOLOGY:

SAMPLE COLLECTION

The Department of Oral and Maxillofacial surgery, Vivekanandha Dental College for Women sourced 75 freshly extracted mandibular premolars with single canal [Fig 1]. These were extracted due to poor periodontal prognosis and orthodontic reasons. X-ray were taken in both buccolingual [Fig 2] and mesiodistal [Fig3] directions to confirm the presence of single canal.

Infection Control protocol:

Occupational Safety and Health Administration (OSHA) and Centre for disease Control and Prevention (CDC) recommendations and guidelines[64, 65] were followed for handling the extracted teeth. Handling of teeth was always done using gloves, mask and protective eyewear.

1. Teeth were cleaned of any visible blood and gross debris.
2. Distilled water was used in wide mouth plastic jars for initial collection.
3. Teeth were immersed in 10% formalin for 7 days, following which the liquid was discarded and the teeth were transferred into separate jars containing distilled water.
4. The initial collection jars, lids and the gloves employed were discarded into biohazard waste receptacles.
5. As and when the teeth were required, they were removed from the jars with cotton tweezers and rinsed in distilled water.

INCLUSION AND EXCLUSION CRITERIA:

Inclusion Criteria:

- Completely formed teeth with intact apices
- Teeth without anatomical variations
- Teeth without caries and root canal fillings
- Teeth with single canal which are fully patent.

Exclusion Criteria:

- Fractured roots
- Teeth with multiple roots
- Open apices
- Calcifications in the canal
- Root resorption and cracks on the surface

PROCEDURE:

Removal of external residual tissues:

The residual tissues on the surface of the teeth were removed and were stored in 2.5% NaOCl solution for 10 minutes. Calculi were removed using hand scalers from the external surfaces and they were again stored in distilled water.

Root canal therapy:

The collected samples were decoronated [Fig 4] with a diamond disc to length of 14 ± 1 mm, measured with the help of a calliper. Access cavity preparation was done with copious water using high speed diamond burs on each teeth. A #10 K file

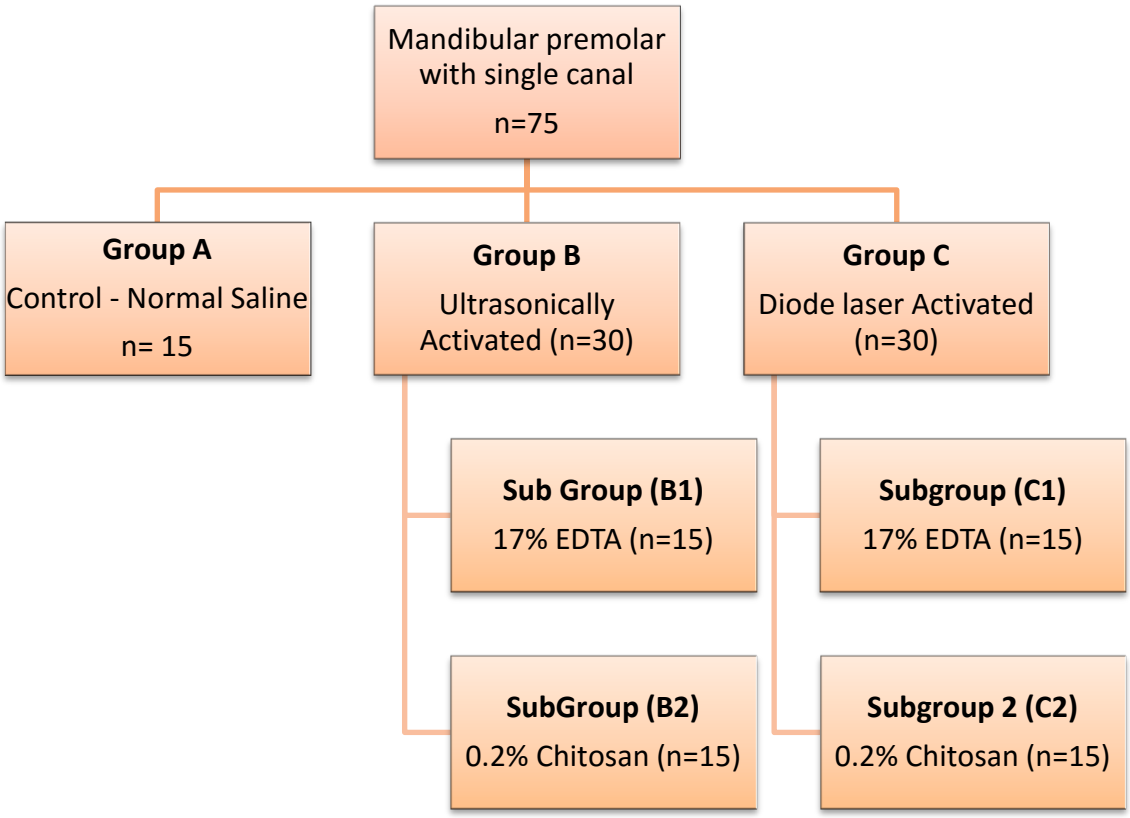
was inserted in the root canal till it was visible at the apical end of the root. Working length determination was done by reducing 1 mm from this measurement.

Pro Taper Universal rotary file was used for canal preparation. To simulate the clinical conditions the apices were sealed with sticky wax. A #20 K file was used for instrumentation of the canal after which it was instrumented up to size F3 ProTaper universal rotary files. 2ml of 3% NaOCl was used for irrigation after using each file. The irrigating solutions were delivered using a 27 gauge needle which was placed 1mm short of the measured working length. Finally, for the flushing out of debris 3ml of 3% NaOCl was used followed by a final rinse with distilled water.

0.2% Chitosan preparation:

Electronic weighing device was used to measure 0.2g of low molecular weight Chitosan. The solution was prepared by dissolving 0.2g of Chitosan[66] in 100 mL of 1% acetic acid [Fig6]. A heated magnetic stirrer [Fig 7] was used to agitate this solution for 2 hours to obtain a homogenous clear solution.[67]

Grouping of Samples:



Group A (Control) – Normal Saline

1ml of Normal saline was used to flush the canals for 1 minute followed by flushing the canal with 3 ml of 3% NaOCl.

Group B1 – Ultrasonically activated EDTA

1ml of EDTA was used as a final flush to irrigate the canals and passive ultrasonic activation was done with #20 U file [Fig8, 10] followed by flushing the canal with 3ml of 3% NaOCl.

Group B2 – Ultrasonically activated Chitosan

Final flush of 1ml of 0.2% Chitosan was used to irrigate the canals and then passive ultrasonic activation was done using #20 U file [Fig 8, 10] for 1 minute, followed by flushing the canal with 3ml of 3% NaOCl.

In groups B1 and B2 the U file was placed into the canal so that it was 1mm short of the measured working length.

Group C1 – Diode laser activated EDTA

0.8ml of 17% EDTA was used to irrigate the canal for 40 seconds and diode laser [Fig 9, 11] was used to activate the remaining 0.2ml for 20 seconds. The treatment was undertaken for four passes of each 5 seconds. Each pass was done at a fibre withdrawal rate of 1mm/second. A fiberoptic tip measuring 200-300µm, 970±15nm, with a power of 2W was used for laser activation of the canal up to the working length. In a helicoid movement the tip was withdrawn to the coronal region and reintroduced to the apical region for an irradiation cycle of 20 seconds, followed by 3ml of 3% NaOCl.

Group C2 – Diode laser activated Chitosan

0.8ml of 0.2% Chitosan was used to irrigate the canal for 40 seconds and diode laser [Fig 9, 11] was used to activate the remaining 0.2ml for 20 seconds. The treatment was undertaken for four passes of each 5 seconds. Each pass was done at a fibre withdrawal rate of 1mm/second. A fiberoptic tip measuring 200-300µm, 970±15nm, with a power of 2W was used for laser activation of the canal up to the working length. In a helicoid movement the tip was withdrawn to the coronal region

and reintroduced to the apical region for an irradiation cycle of 20 seconds, followed by 3ml of 3% NaOCl.

5ml of distilled water was used as a final flush in all the samples to terminate the action of the other irrigants used. Scanning electron microscopic examination [Fig 12, 13] was carried out after the samples were dried and prepared.

SEM Analysis:

Scanning electron microscopic analysis [Fig 4] was carried out to analyse the efficacy of the irrigants used on the smear layer removal from the dentinal walls. A diamond disc was used to make longitudinal grooves on the buccal and lingual surfaces of all the roots without penetration of the canal and a chisel was used to split them into two halves. The sample of each group were placed on metal stubs, dried and coated with gold before examination under Scanning electron microscope (Sigma FE-SEM, Zeiss) at X2000and X5000 magnification for verification of the patency of the dentinal tubules. Photographs were taken at the at coronal, middle and apical segments of the root canals and scoring was done using the Gutman et at[21] rating system.

Gutmann rating system for remaining smear layer scores

Score	Criteria
1	Little or no smear layer; covering<25% of the specimen; most tubules were visible and patent, or almost complete laser melting
2	Little to moderate or patchy mounts of smear layer; covering 25–50% of the specimen; many tubules visible and patent, or laser melting
3	Moderate amounts of scattered of aggregated smear layer; covering 50–75% of the specimen; minimal to no tubule visibility or patency, or scattered laser melting
4	Heavy smear layer covering>75% of the specimen; no tubule orifices were visible or patent; or no visible laser melting

Fig 1: 75 Single rooted mandibular premolar

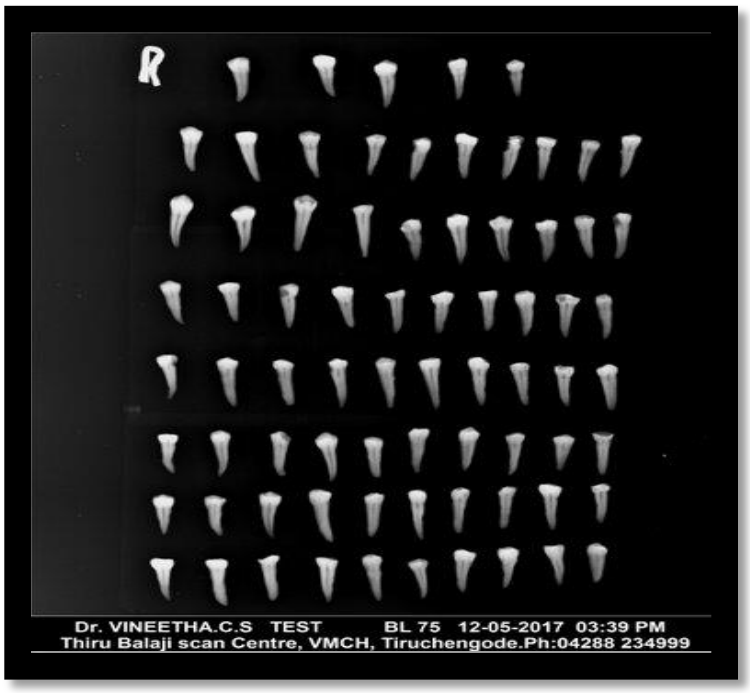


Fig 2: Verification of single canal: X- ray images. (Buccolingual)

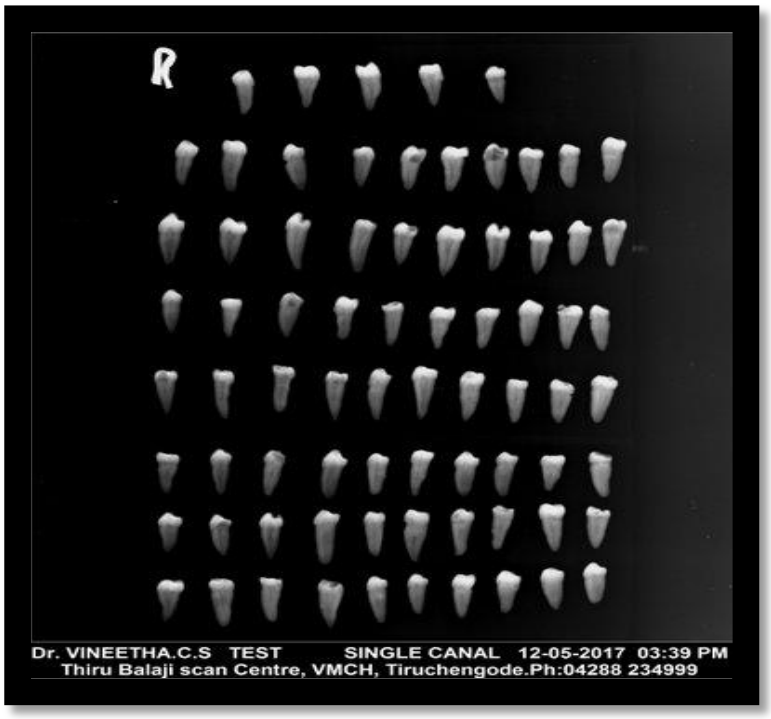


Fig 3: Verification of single canal: X- ray image (Mesiodistal)

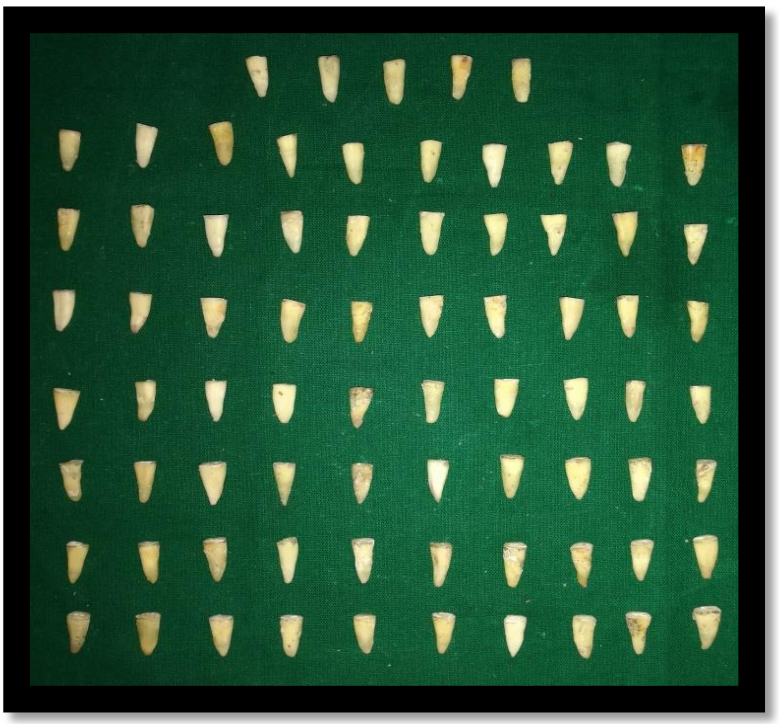


Fig 4: Decoronated samples

Fig 5: Armamentarium

Fig 6: Chitosan and Acetic acid

Fig 7: Heated Magnetic Stirrer

Fig 8: Ultrasonic unit

Fig 9: Diode Laser

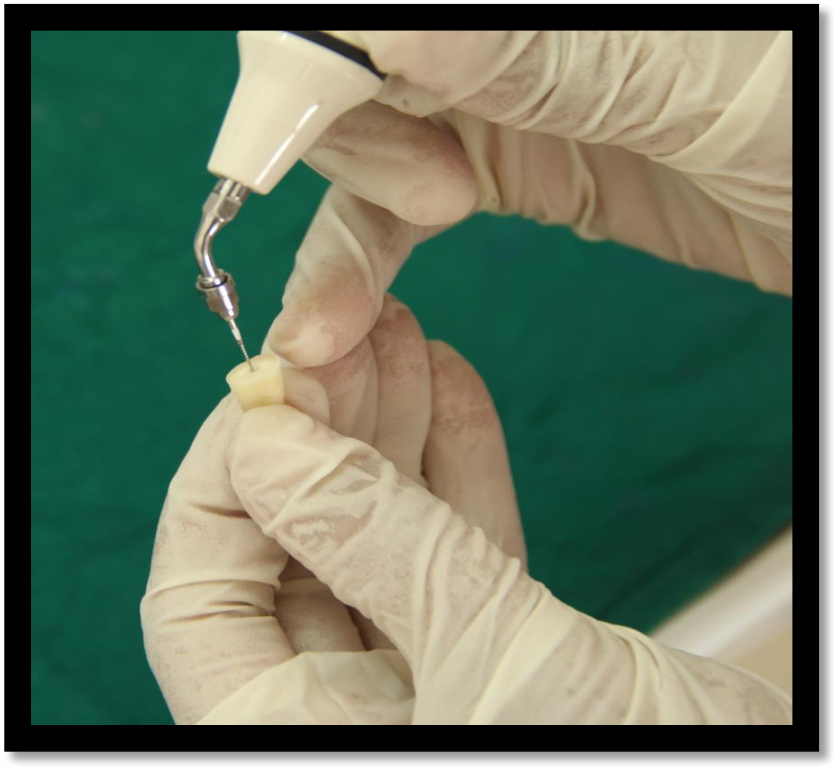


Fig 10: U File used for agitation of irrigant

Fig 11: Diode Laser used for agitation of irrigant

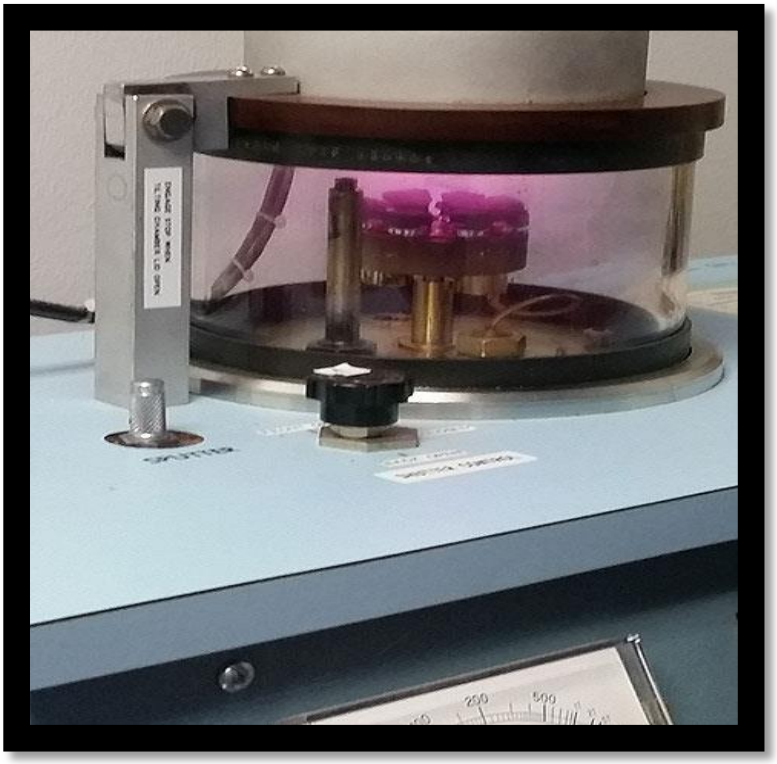


Fig 12: Magnetron Sputtering Coater

Fig 13: Samples secured on metal stubs and sputter coated with gold

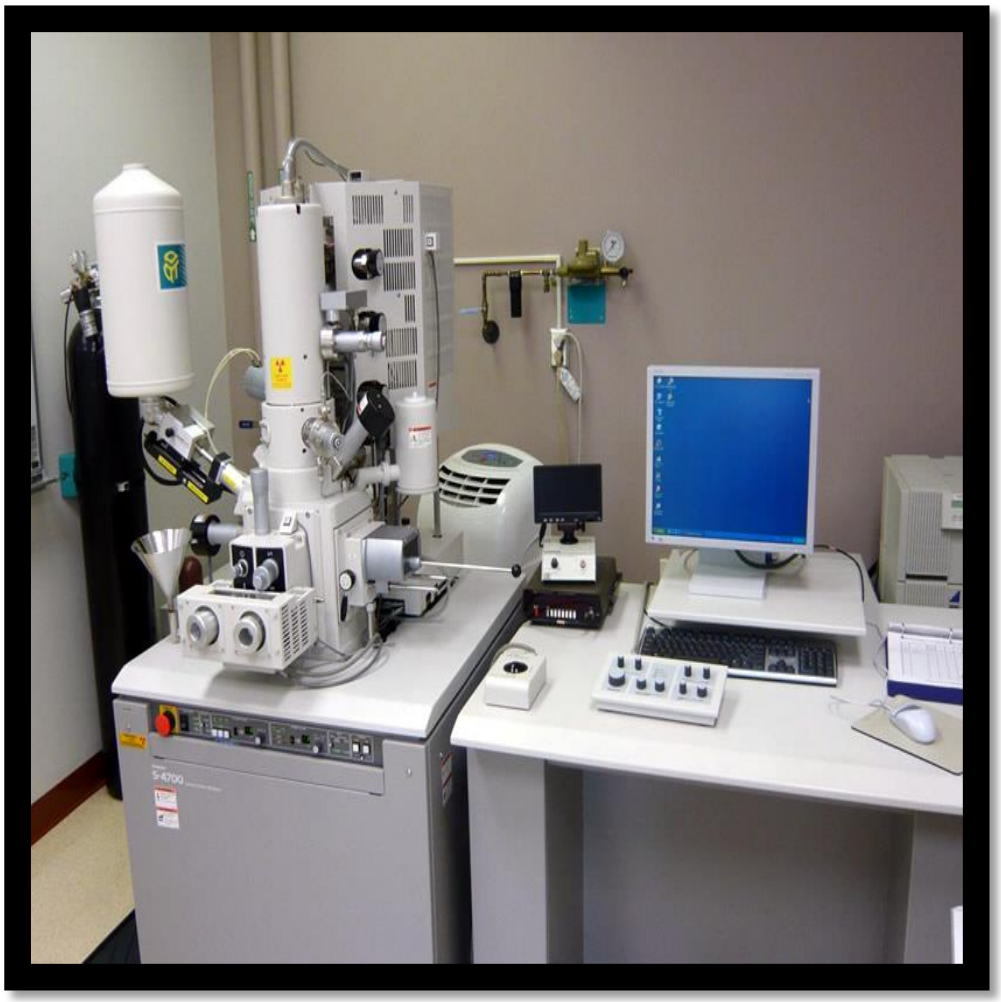


Fig 14: Scanning Electron Microscope

GROUP A (NORMAL SALINE)

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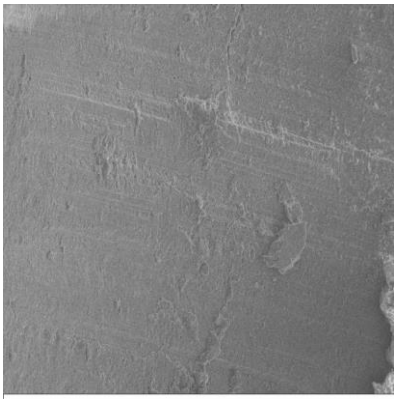
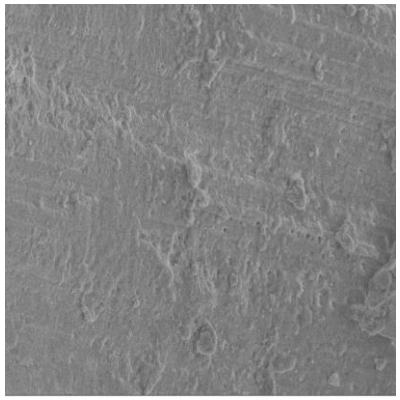
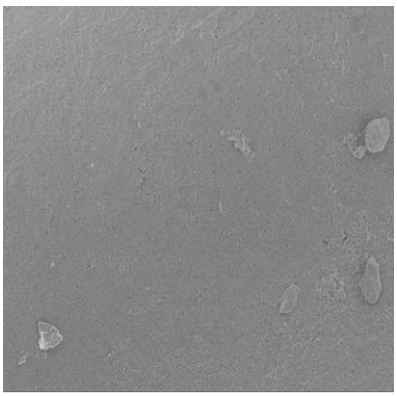
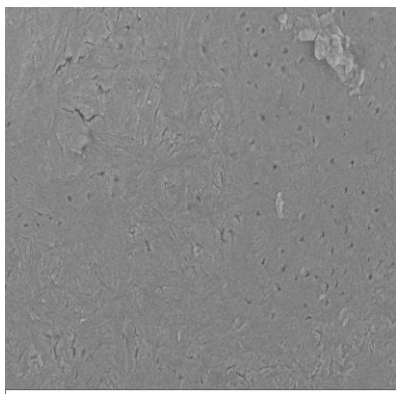
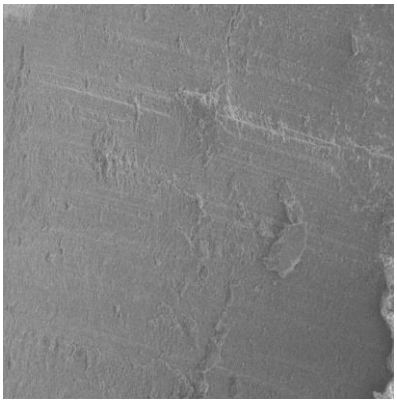
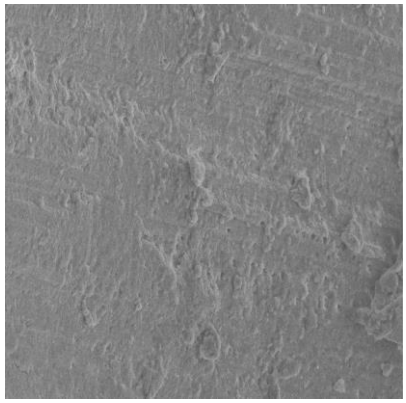
CORONAL		
MIDDLE		
APICAL		

GROUP B1 (ULTRASONICALLY ACTIVATED –EDTA)

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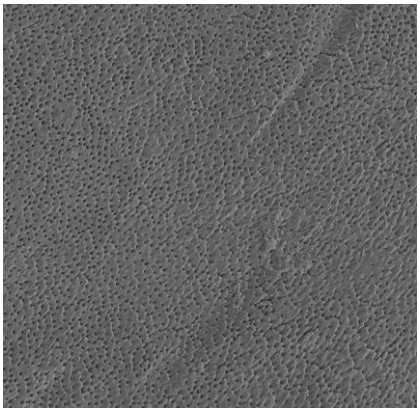
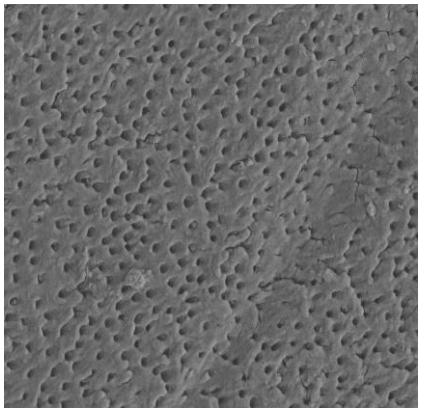
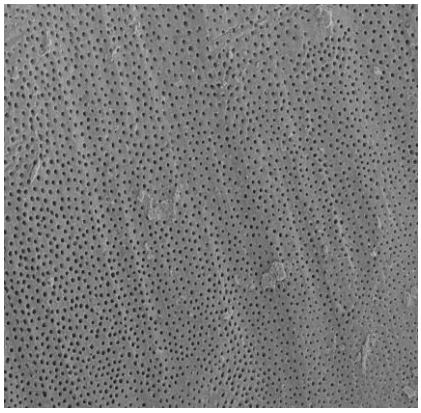
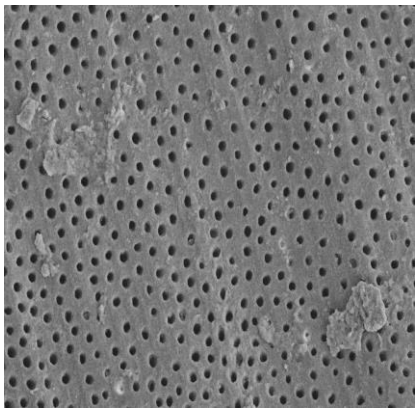
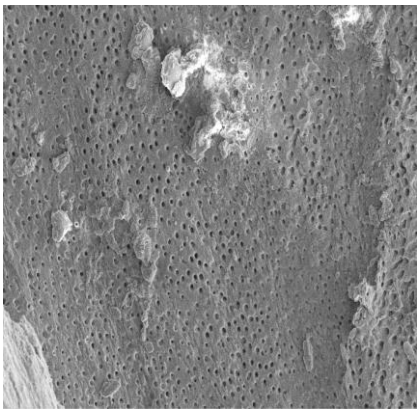
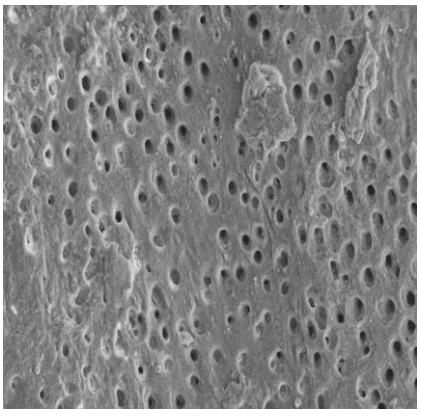
CORONAL		
MIDDLE		
APICAL		

GROUP B2 (ULTRASONICALLY ACTIVATED – CHITOSAN)

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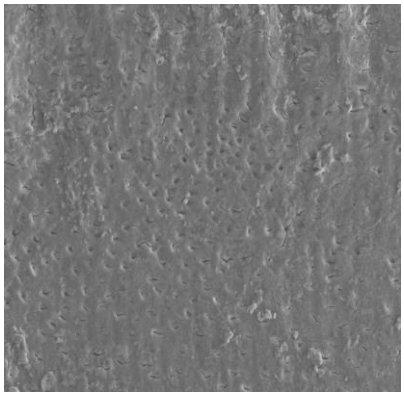
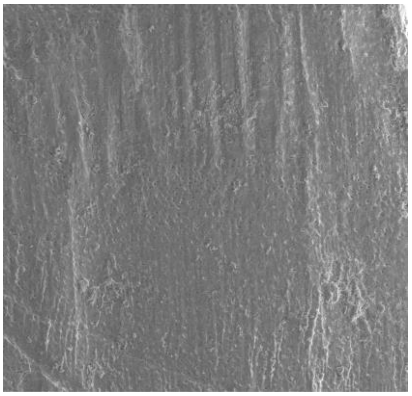
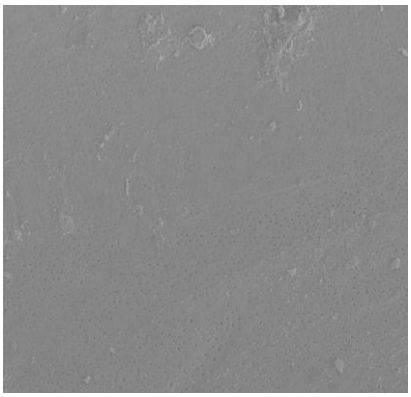
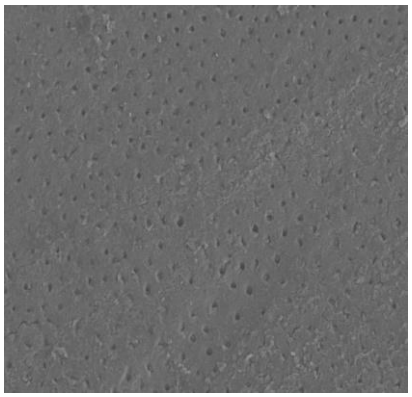
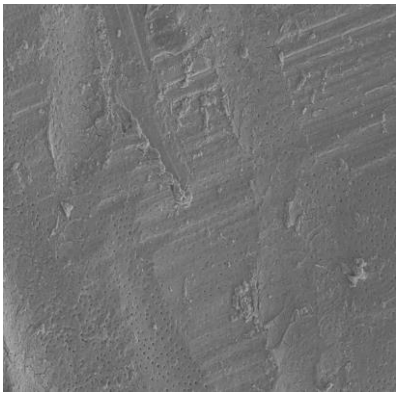
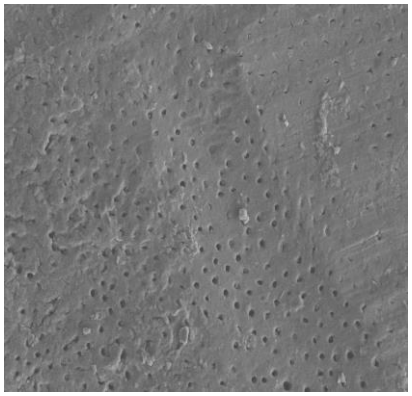
CORONAL		
MIDDLE		
APICAL		

GROUP C1 (DIODE LASER ACTIVATED – EDTA)

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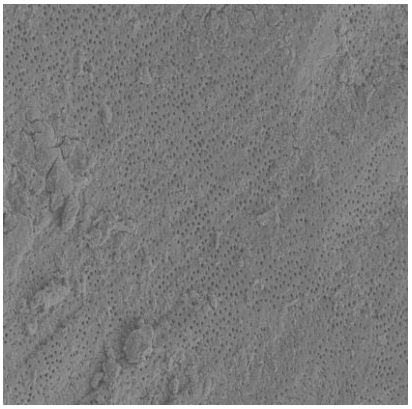
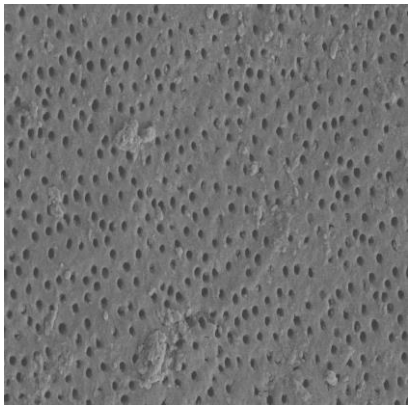
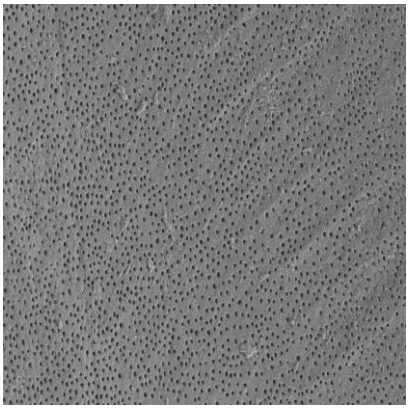
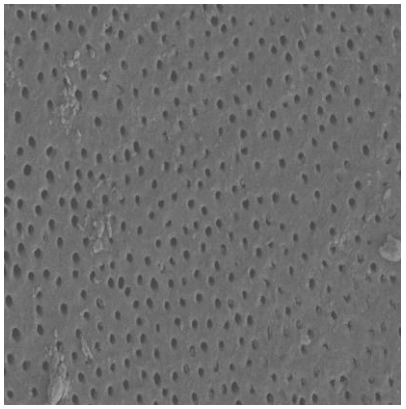
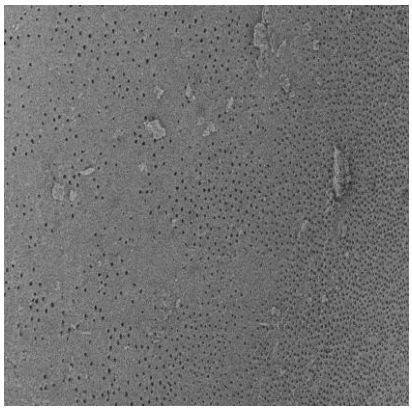
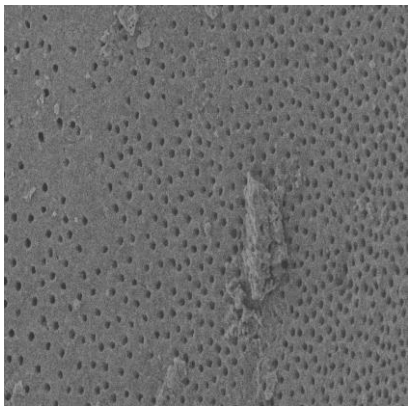
CORONAL		
MIDDLE		
APICAL		

GROUP C2 (DIODE LASER ACTIVATED – CHITOSAN)

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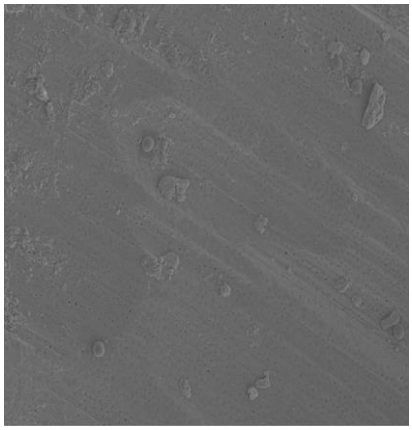
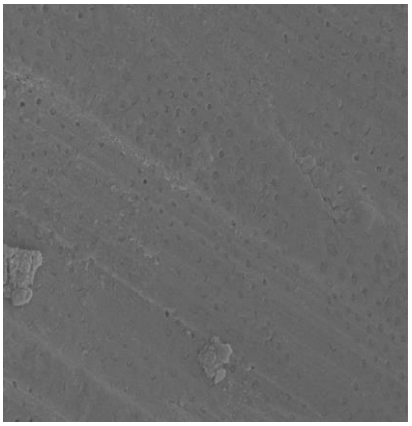
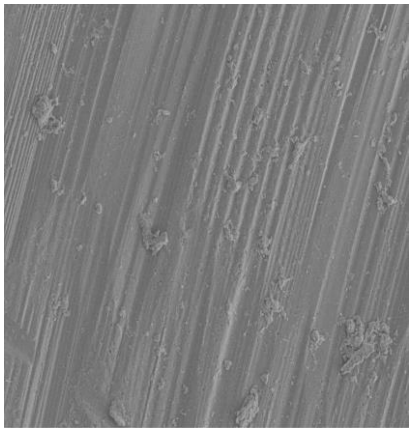
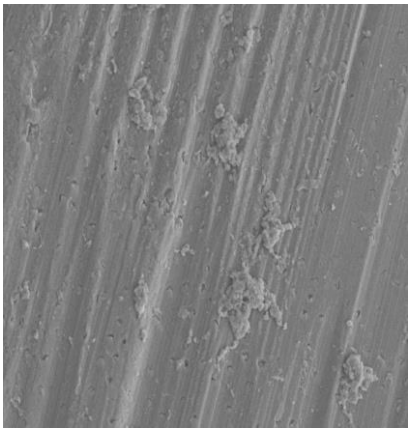
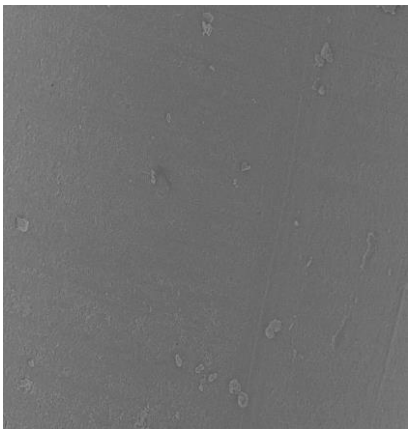
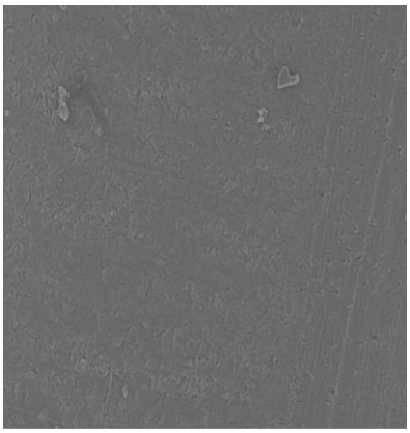
CORONAL		
MIDDLE		
APICAL		

RESULTS

RESULTS

The mean values of the remaining smear layer scores were tabulated (Table 1). Analysis of the data was done using One-way analysis of variance (ANOVA), using the SPSS version 20. The values were considered statistically significant when P value < 0.05.

There was statistically significant difference among all the tested groups except among Group B2 and C1 in the apical third, which had no significant difference (Table 2).

Table 1: Scores of mean remaining smear layer among various groups

Area Recorded	**Group A (Normal Saline)**	**Group B1 (Ultrasonics + EDTA)**	**Group B2 (Ultrasonics + Chitosan)**	**Group C1 (Diode Laser + EDTA)**	**Group C2 (Diode Laser + Chitosan)**
Coronal third	3.2	1.4	2.4	1	2.2
Middle third	3.33	1	2.4	1.2	2.8
Apical third	3.8	1	1.4	1.4	2
Total	10.33	3.4	6.2	3.6	7

Table 2 : Intra group comparison of remaining smear layer scores at various levels

GROUPS	CORONAL			MIDDLE			APICAL			OVERALL		
	Mean difference	**P value**	**Sig**	**Mean difference**	**P value**	**Sig**	**Mean difference**	**P value**	**Sig**	**Mean Difference**	**P Value**	**Sig**
A vs B1	1.800	0.000	S	2.333	0.000	S	2.800	0.000	S	6.933	0.000	S
A vs B2	0.800	0.000	S	0.9333	0.000	S	2.400	0.000	S	4.133	0.000	S
A vs C1	1.800	0.000	S	2.333	0.000	S	2.800	0.000	S	6.933	0.000	S
A vs C2	0.800	0.000	S	0.933	0.000	S	2.400	0.000	S	4.133	0.000	S
B1 vs B2	-1.000	0.000	S	-1.400	0.000	S	-0.400	0.400	S	-2.800	0.000	S
B1 vs C1	0.000	1.000	NS	0.000	1.000	NS	0.000	1.000	NS	0.000	1.000	NS
B1 vs C2	-1.000	0.000	S	-1.400	0.000	S	-0.400	0.004	S	-2.800	0.000	S
B2 vs C1	1.000	0.000	S	1.400	0.000	S	-0.400	0.400	S	2.800	0.000	S
B2 vs C2	0.000	1.000	NS	0.000	1.000	NS	0.000	1.000	NS	0.000	1.000	NS
C1 vs C2	-1.000	0.000	S	-1.400	0.000	S	-0.400	0.004	S	-2.800	0.000	S

Coronal third – C1 > B1 > C2 > B2 > A

Middle third – B1 > C1 > B2 > C2 > A

Apical third – B1 > B2 = C1 > C2 > A

Overall – B1 > C1 > B2 > C2 > A

Scanning Electron Microscopy analysis of the experimental specimens at 2000x and 5000x magnifications revealed that among the tested specimens, the efficacy of smear layer removal of Diode laser activated EDTA was highest at the coronal third. In the middle and apical third, ultrasonically activated EDTA had the highest efficacy. The dentinal tubule orifices were patent with clearly demarcated boundaries in both the groups. Normal saline had the least efficacy as compared to the other groups throughout the length of the specimen which showed occluded orifices on the dentin surface with florid debris.

DISCUSSION

DISCUSSION

The disinfection of dentin walls using irrigants is adversely affected in the presence of smear layer by blocking them from entering dentinal tubules[2]. It also adversely affects sealer penetration and increases microleakage following obturation leading to increased intra-canal microflora [68, 69].

Hence, to enhance sealer penetration and a fluid tight seal it is necessary to remove the smear layer. There has been an increasing interest in developing new irrigating solutions due to the limitations of the presently available ones.

Chitosan is a natural, cationic aminopolysaccharide copolymer of glucosamine and N-acetylglucosamine obtained by the alkaline, partial deacetylation of chitin. It is obtained from shells of crustaceans and shrimps[44]. It has biocompatibility, biodegradability, bioadhesion as well as antimicrobial activity[70]. It can also chelate various metal ions such as Fe2+, Co2+, Mg2+, Zn2+ and Cu2+ respectively) in acid conditions[71]. As it is hydrophilic, it is adsorbed on the root canal wall[72].

In this study, mandibular premolars with single canal were decoronated at the CEJ for standardisation. ProTaper universal system up to size F3 was used for preparation of the root canal since it provides a uniform preparation. Specimens were randomly divided into 3 groups and 2 subgroups – Group A- Normal Saline (Control), Group B – Ultrasonic activation, Group C – Diode Laser activation, Group B and C were subdivided into (B1 and C1) 17% EDTA and (B2 and C2) 0.2% Chitosan.

According to Silva et al[48] the efficacy of 0.2% chitosan on the removal of smear layer was better than that of 1% acetic acid. This is important because in the present study 1% acetic acid was used in the preparation of chitosan solution.

Chitosan citrate produces more amount of dentinal erosion when compared to chitosan acetate when used as an irrigant for smear layer removal [48].

A.M.Darrag[54] studied the ability of ability of 17% EDTA, 10% CA, MTAD, and 0.2% chitosan solutions to remove the smear layer and concluded that 0.2% chitosan was better, but there is no significant difference among them.

The effect of smear layer removal by Chitosan was compared to that of EDTA because EDTA is has been accepted as a gold standard for removal of smear layer[2, 48]

A combination of NaOCl and EDTA has been successfully used in debridement and for enlarging narrow and obstructed canals. Fraser in 1974[73], found that in the apical third the chelating ability of EDTA was minimal and it also caused root dentine erosion. EDTA also has limited antimicrobial activity compared to NaOCl. To minimise the harmful effects of EDTA the search continues for a newer material which is more biocompatible with enhanced antimicrobial effect.

Chitosan removes the inorganic portion of the smear layer due to its chelating ability, but its effect for endodontic application has not been widely explored. But this property has been used for the recovery of metal ions and purification of drinking water[74].

In the present study, Chitosan solution preparation was done using 1% acetic acid. According to Silva et al[48], it was attributed that the chelating ability of Chitosan was because of its own properties and not by 1% acetic acid. Thus, we could deduce that the chelating behaviour of Chitosan favoured its smear layer removal.

In the current study, Ultrasonics and 970nm Diode laser were used as an adjuncts. This was because several studies showed that the addition of ultrasonics

increased the smear layer removing by enhancing the penetration of irrigating solution into the narrow apical regions of the root canals[1, 75, 76, 77].

When endodontic files are used in the handpiece, the files oscillate along the longitudinal axis of the instrument, with maximal amplitude occurring at the antinodes and minimal oscillation at the nodes[78]. The file oscillations are primarily responsible for the production of acoustic streaming (vortex like motion). Acoustic streaming may also be associated with the occurrence of cavitation which enhances smear layer removal[79, 80].

Laser activation of irrigating solutions enhances the efficacy of the irrigating solution by the absorption of laser energy. This causes formation of vapour bubbles followed by collapse of these bubbles, acoustic streaming, which finally leads to cavitation[50, 56].

According to Walmsley et al, the smear layer removal at the apical third was found to be the least because of the constriction in the root canal, which restricted the oscillation of the ultrasonic tip. The apical part is the most affected due to attenuation of oscillation because the amplitude is greatest at the tip of the instrument[78]. This was in accordance with the current study in which EDTA and Chitosan which showed effective smear layer removal from coronal third.

Comparing the overall efficacy of various combinations used in this study, Group B1 (EDTA+ Ultrasonics) produced better smear layer removal than Group B2 (EDTA+Diode Layer), which in turn was better than Group C1 and C2 i.e., a combination of Chitosan with Ultrasonics and diode laser respectively. Group A had the least efficacy in the removal of smear layer.

In Group A (Normal Saline) there was thick smear layer all through the length of the root canal which is in accordance to a study conducted by Mensudar Rathakrishnan et al[81].

In the coronal third, a combination of EDTA with diode laser had the least remaining smear layer score. This was similar to the study Neelakantan et al, in which diode laser was found to be better than ultrasonics in the disinfection of dentinal tubules[56].

Arslan et al evaluated the activation of 15% EDTA using 808-nm diode laser and concluded that on the removal of smear layer removal and concluded that agitation with diode laser was effective in the removal of smear layer. This was in accordance with the present study where EDTA activated with Diode laser had the greatest efficacy of smear layer removal [50].

In the middle and apical 3rd, Group B1 i.e., a combination of ultrasonics with EDTA was the most effective in the removal of smear layer. In the middle 3rd, following Group B1, Group C1 i.e., diode laser activated EDTA was better than Group B2 i.e., Ultrasonically activated Chitosan , which in turn was better than Group B2 and C2 i.e., Chitosan which was activated with Ultrasonics and Diode laser respectively. In the apical 3rd, Group B1 i.e., a combination of ultrasonics with EDTA had the maximum efficacy of smear layer removal. This was similar to a study conducted by Amin et al [58]. Following this, the efficacy of Group B2 i.e., ultrasonically activated Chitosan was better in the removal of smear layer than Group A i.e., Normal saline and Groups B2 and C1 i.e., Ultrasonically activated EDTA and Diode laser activated Chitosan produced similar results. These results were not in accordance with the study conducted by A. M. Darrag et al[54] which showed 0.2%

Chitosan to be more effective than 17% EDTA and 10% Citric acid. This can be due to the use of Ultrasonics as adjunct in the present study, which shows better results when used in combination with EDTA.

Comparing the overall efficacy of various combinations used in this study, Group B1 (EDTA+ Ultrasonics) produced better smear layer removal than Group B2 (EDTA+Diode Layer), which in turn was better than Group C1 and C2 i.e., a combination of Chitosan with Ultrasonics and diode laser respectively. Group A had the least efficacy in the removal of smear layer.

According to a several recent studies, a combination of Chitosan- EDTA (1:1) can perform as a root canal disinfectant and can also be used in the removal of smear layer [82, 83, 84]. EDTA potentiates the antibacterial activity of Chitosan and facilitates the entry of Chitosan into bacterial cell, this combination is known to restrain the growth of microorganisms by enzyme inhibition[83, 84].

Hence, from the current study, it can be inferred that the combination of Diode laser with EDTA had the maximum efficacy in the coronal 3rd and a combination of Ultrasonics with EDTA had the maximum ability of smear layer removal in the middle and apical 3rd. Further studies i) using a combination of EDTA and Chitosan, ii) using higher concentration of Chitosan and iii) activation of irrigants using lasers, ultrasonics and newer adjuncts and more in vivo studies need to be carried out to support the results of the current study and to achieve clinical success.

Limitations:

- Being an in-vitro study, the results cannot be directly correlated to the clinical situations.
- Further studies are needed to evaluate the concentration and efficacy of chitosan required for smear layer removal.
- In the present study the ability of smear layer removal was only evaluated, other properties such as biocompatibility and antibacterial efficacy were not assessed.

SUMMARY &CONCLUSION

SUMMARY AND CONCLUSION

This study was to compared the ability of Ultrasonically activated and Laser activated EDTA and Chitosan on smear layer removal by using Scanning Electron Microscope.

Seventy five mandibular premolars with single canal were collected. These were decoronated at the level of cementoenamel junction. Cleaning and shaping was carried out using Protaper rotary file system up to F3 and the samples were divided randomly into 3 groups and 2 subgroups based on the irrigation protocol.

Group A (Normal saline), Group B1 (ultrasonically activated –EDTA), B2 (ultrasonically activated Chitosan), Group C1 (Diode laser activated – EDTA) and C2 (Diode laser activated – Chitosan).

After following the irrigation protocol for each group, the samples were sectioned longitudinally using a diamond disc and Scanning electron Microscopic analysis was carried out to study the surface morphology of root dentin.

The remaining smear layer scores were obtained based on Gutmann's criteria and the data were tabulated. Statistical analysis was carried out using one- way analysis of variance using SPSS software version 20 and results were obtained.

Under the limitations of the present study, Diode laser activated EDTA had the highest efficacy of smear layer removal at the coronal third. In the middle and apical third, ultrasonically activated EDTA had the highest efficacy. Normal saline had the least efficacy as compared to the other groups throughout the length of the specimen.

Hence from the results of the present study, it can be concluded that using Chitosan may be an alternative to EDTA, in the removal of smear layer considering the drawbacks of EDTA but further studies using higher concentrations of Chitosan and in vivo studies need to be carried out to support the results of the present study.

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Dr. R. Jagan Mohan	Clinician	Dr. N. Meenakshiammal	Medical Scientist
Dr. B.T. Suresh	Scientific Member	Dr. R. Natarajan	Scientific Member
Dr. Sachu Philip	Scientific Member	Mr. Kamaraj	Lay Person

No: VDCW/IEC/19/2016 Date: 05.11.2016

TO WHOMSOEVER IT MAY CONCERN

Principal Investigator: Dr. Vineetha.C.S.

Title: Electron Microscopic Comparative Analysis of Smear layer removal by Ultrasonically activated and Diode Laser activated EDTAand Chitosan- An Invitro study.

Institutional ethics committee thank you for your submission for approval of above proposal .It has been taken for discussion in the meeting held on 25 .10.16.The committee approves the project and it has no objection on the study being carried out in Vivekanandha Dental College For Women.

You are requested to submit the final report on completion of project. Any case of adverse reaction should be informed to the institutional ethics committee and action will be taken thereafter.

CHAIRMAN
INSTITUTIONAL ETHICS COMMITTEE
VIVEKANANDHA
DENTAL COLLEGE FOR WOMEN
Elayampalayam-637 205
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